BACTERIOLOGY.—*Incidence of leptospirosis among dogs in Honolulu as determined by serological agglutination tests.*[1] Joseph E. Alicata and Virginia Breaks, Honolulu, T. H.

Recent reports of the widespread occurrence of leptospirosis among dogs in the continental United States have caused considerable veterinary and public-health interest in this disease. Geographically, the cases reported involve 14 states: Alabama, California, Connecticut, Georgia, Louisiana, Massachusetts, Maryland, Michigan, New Jersey, New York, Ohio, Pennsylvania, Virginia, and Wisconsin. Cases have been reported also from the District of Columbia and Puerto Rico (1). Serological studies have shown 34.0 percent canine infection in some sections of California (4), 11.8 percent in New York (4), and 38.1 percent in Pennsylvania (5). These and other reports have led to the conclusion that probably 25 to 50 percent of the dogs in the United States are temporary or permanent carriers of the causative organisms *Leptospira canicola* and *L. icterohemorrhagiae* (2).

The present paper, reporting on positive leptospiral sero-reactions, presents additional information regarding the geographical distribution and incidence of canine leptospirosis. The presence of this disease in dogs became suspected following recognition of local human and murine leptospirosis in 1937 (3), and from communications received from local veterinarians regarding the existence of undiagnosed cases of acute jaundice. The first findings of *Leptospira* agglutinins in the sera of local dogs were made in 1940 on the sera of 7 out of 11 dogs submitted for examination by Dr. L. C. Moss, veterinarian, to Dr. K. F. Meyer, Hooper Foundation, San Francisco, Calif.

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Methods.—Sera were collected from 23 dogs (nos. 1–23) submitted to a veterinary hospital for various causes and 77 (nos. 24–100) obtained at random from a local dog pound. In each case the blood was withdrawn from the femoral vein. Only one test was conducted on each animal. The microscopic agglutination test, using fresh formalin-killed antigens, was used. The porcelain-plate method as described by Meyer, Stewart-Anderson, and Eddie (4) was adopted. Each of the sera was tested for agglutinins against the canicola strain, *L. canicola*, and the classical strain, *L. icterohemorrhagiae*. A series of dilutions of serum was made with Verwoort-Schüffner buffer solution. The final dilutions in the plates ranged from 1:10 to 1:30,000. The clumps of agglutinated organisms were ascertained by the use of a darkfield. Since agglutination in low dilutions is considered doubtful, only serum positive in a dilution of 1:100, or greater, is regarded in this study as significant.

Results and interpretation of the serological agglutination tests.—As shown in Table 1 and summarized in Table 2, of the 100 dogs examined 20 gave stronger sero-agglutination reactions to *L. icterohemorrhagiae* than to *L. canicola*, as follows: 1 in 1:100, 15 in 1:300, 2 in 1:1000, 1 in 1:10,000, and 1 in 1:30,000. In addition, 19 sera reacted more strongly to *L. canicola*, as follows: 1 in 1:100, 7 in 1:300, 5 in :1,000, 1 in 1:10,000, and 5 in 1:30,000. Those cases in which the titer was between 1:100 to 1:1000 and the animals appeared normal were considered as possible latent infections; those that showed illness, as in nos. 1, 2, 9, 13, and 21, might have represented an early stage of the disease and not sufficient time had elapsed for agglutinins to develop in larger amounts. The 8 cases reported (nos. 17, 3, 27, 12, 35, 62, 78, and 88) in which the agglutination titer was between 1:10,000 and 1:30,000 are regarded as active clinical cases. Of these, the 2 dogs (nos. 17 and 3)

reacting positive to the classical strain showed jaundice whereas the other 6 dogs reacting positive to the canicola strain were anicteric and showed, in most cases, general malaise, muscular tremor, and dehydration.

Through the use of culture methods (Verwoort's medium), leptospirae were isolated from the kidneys of two dogs suspected of having died of canicola fever. One of these dogs, just before death, showed a sero-agglutination titer of 1:300,000 for *L. canicola* and 1:30,000 for *L. icterohemorrhagiae*. The other dog, just before death, showed a sero-agglutination titer of 1:10,000 for *L. canicola* and 1:1,000 for *L. icterohemorrhagiae*.

Discussion.—The results of this study indicate that 39 percent of the dogs examined had or were passing through a case of leptospirosis. Of these, about one-half showed infection with the classical strain and the other half with the canicola strain. These findings differ from reports of surveys conducted in the continental United States where the canicola strain has usually been found most common. In San Francisco, of 59 dogs examined 3 reacted to the classical strain and 33 to the canicola strain (4); in New York, of 111 dogs examined 3 reacted to the classical strain and 10 to the canine strain (4); in Pennsylvania, of 105 dogs examined canicola strain was found to be three times as frequent as the classical strain (5). On the other hand, Meyer and coworkers (4) found 10 out of 12 dog sera from Detroit, Mich., positive for the classical strain; the dogs, in this case, were reported to be in some way connected with cases of human Weil's disease.

The epizootiological relationships of canine and murine leptospirosis has not been definitely established. In San Francisco, Meyer and coworkers (4) found a low incidence of infection with *L. icterohemorrhagiae* among dogs in spite of the fact that 35 percent of the rats in the same locality were found to carry this organism. Of a series of 500 rats trapped at random in Honolulu, silver-stained sections of kidneys of these animals revealed only 13 or 2.6 percent infection with leptospirae. All these positive rats were trapped in localities near fresh-water streams. Kidney emulsion of these rats produced clinical leptospirosis with jaundice when inoculated into young mice and guinea pigs. These findings point out a low relationship between the incidence of murine and canine leptospirosis. Infection of dogs with the classical strain appears therefore to be brought about by intercanine association in the same way that the canicola infection takes place among dogs. The canicola strain as far as is known is not found in rats.

Summary.—Microscopic agglutination tests, using fresh formalin-killed *Leptospira canicola* and *L. icterohemorrhagiae* as antigens, have been conducted on the sera of 100 dogs from Honolulu. Of these, 20 percent of the sera gave stronger agglutination reactions to *L. icterohemorrhagiae* than to *L. canicola* and 19 percent reacted more strongly to *L. canicola*.

Leptospirae were recovered from two dogs suspected of having died of canicola fever.

Out of 500 rats examined in Honolulu, 2.6 percent were found to harbor leptospirae. This low incidence points out little epizootiological relationship between murine and canine infections. The disease in dogs with the classical strain of *Leptospira* is believed to be brought about through intercanine associations as in the case of the canicola strain.

LITERATURE CITED

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TABLE 1.—POSITIVE LEPTOSPIRA AGGLUTINATION REACTIONS NOTED IN THE SERA OF 39 OUT OF 100 DOGS EXAMINED; FORMALINIZED CULTURES OF L. ICTEROHEMORRHAGIAE AND L. CANICOLA USED

Dog No.	Titer of sero-reaction: First line for *L. icterohemorrhagiae;* second line for *L. canicola*								Clinical observations
	1:10	1:30	1:100	1:300	1:1,000	1:3,000	1:10,000	1:30,000	
42	+++ +++	++ +	++ 0	0 0	0 0	0 0	0 0	0 0	Normal
1	++ 0	++ 0	++ 0	+ 0	0 0	0 0	0 0	0 0	Icteric
2	+++ 0	+++ 0	+++ 0	++ 0	0 0	0 0	0 0	0 0	General malaise
9	+++ +++	+++ +	++ 0	+ 0	0 0	0 0	0 0	0 0	Bloody urine
10	++ 0	++ 0	++ 0	+ 0	0 0	0 0	0 0	0 0	Dermatitis
13	+++ +++	+++ ++	++ +	+ 0	0 0	0 0	0 0	0 0	Coughing, subnormal temperature
19	++++ +	+++ +	++ 0	+ 0	0 0	0 0	0 0	0 0	Normal
51	+++ ++	+++ ++	++ +	+ 0	0 0	0 0	0 0	0 0	Normal
53	+++ +	+++ 0	++ 0	+ 0	0 0	0 0	0 0	0 0	Normal
59	+++ ++	+++ ++	++ +	+ 0	0 0	0 0	0 0	0 0	Normal
60	+++ +++	+++ ++	++ +	+ 0	0 0	0 0	0 0	0 0	Normal
61	+++ +++	+++ ++	++ +	+ 0	0 0	0 0	0 0	0 0	Normal
69	+++ ++	+++ ++	+ +	+ +	0 0	0 0	0 0	0 0	Normal
74	++++ +++	+++ ++	+++ +	++ +	0 0	0 0	0 0	0 0	Normal
84	++++ ++	+++ ++	++ +	+ 0	0 0	0 0	0 0	0 0	Normal
95	+++ ++	+++ +	++ 0	+ 0	0 0	0 0	0 0	0 0	Normal
68	++++ ++	+++ ++	++ +	+ +	+ 0	0 0	0 0	0 0	Normal
80	++++ ++	+++ ++	+++ +	++ 0	+ 0	0 0	0 0	0 0	Normal
17	++++ +++	++++ ++	+++ ++	+++ ++	++ +	+ +	+ 0	0 0	Icteric; vomiting
3	++++ ++++	++++ ++++	++++ ++++	++++ +++	+++ ++	+++ 0	+++ 0	++ 0	Icteric
64	++ +++	+ +++	0 +	0 0	0 0	0 0	0 0	0 0	Normal
16	+++ +++	++ +++	+ ++	0 +	0 0	0 0	0 0	0 0	Stomatitis

TABLE 1—*Continued*

Dog No.	Titer of sero-reaction: First line for *L. icterohemorrhagiae;* second line for *L. canicola*								Clinical observations
	1:10	1:30	1:100	1:300	1:1,000	1:3,000	1:10,000	1:30,000	
18	++ ++	+ ++	0 ++	0 +	0 0	0 0	0 0	0 0	Normal
29	++ +++	++ ++	+ ++	0 +	0 0	0 0	0 0	0 0	Normal
55	++++ ++++	+++ ++++	++ +++	0 +	0 0	0 0	0 0	0 0	Normal
67	++ +++	+ +++	+ ++	0 +	0 0	0 0	0 0	0 0	Normal
83	+++ +++	++ +++	+ ++	0 +	0 0	0 0	0 0	0 0	Normal
93	++ ++++	+ +++	+ ++	0 +	0 0	0 0	0 0	0 0	Normal
21	++ +++	+ +++	+ ++	+ ++	0 +	0 0	0 0	0 0	Vomiting
37	+++ +++	++ +++	+ ++	0 ++	0 +	0 0	0 0	0 0	Normal
43	+++ ++++	++ +++	++ ++	+ ++	0 +	0 0	0 0	0 0	Normal
46	+ +++	+ +++	0 ++	0 ++	0 +	0 0	0 0	0 0	Normal
57	++++ ++++	+++ +++	++ +++	+ +	+ +	0 +	0 0	0 0	Normal
27	++ ++++	++ ++++	+ +++	+ +++	0 ++	0 +	0 +	0 0	Malaise; dehydration; muscular tremor
12	++++ ++++	++++ ++++	++++ ++++	+++ ++++	+++ ++++	+ ++++	0 +++	0 +	Vomiting; dehydration
35	++ ++++	+ ++++	0 ++++	0 ++++	0 +++	0 +++	0 +++	0 ++	Malaise; dehydration
62	++++ ++++	++++ ++++	++++ ++++	++++ ++++	+++ ++++	+++ ++++	+++ ++++	++ +++	Dehydration; muscular tremor
78	++++ ++++	++++ ++++	+++ ++++	+++ ++++	+ +++	0 ++	0 ++	0 +	Malaise; dehydration
88	++++ ++++	++++ ++++	+++ ++++	+++ ++++	++ ++++	++ ++++	++ +++	+ ++	Malaise; muscular tremor

TABLE 2.—SUMMARY OF THE AGGLUTINATION TITER OF THE 39 POSITIVE DOG SERA

Number reacting more strongly to:	Titer						Total
	1:100	1:300	1:1,000	1:3,000	1:10,000	1:30,000	
L. icterohemorrhagiae.....	1	15	2	0	1	1	20
L. canicola.....	1	7	5	0	1	5	19